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MATRIX PROTEIN COMPOSITIONS FOR DENTIN REGENERATION

FIELD OF INVENTION

The present invention relates to the use of enamel matrix, enamel matrix derivatives and/or enamel matrix proteins for dentin regeneration.

BACKGROUND OF THE INVENTION

Enamel matrix proteins such as those present in enamel matrix are well-known as precursors of dental enamel. Enamel matrix proteins and enamel matrix derivatives have previously been described in the literature to induce enamel formation (US 4,672,032, Slavkin) or binding between different types of mineralised tissue such as cementum and bone (EP-B-0 337 967 and EP-B-0 263 086). Furthermore, enamel matrix proteins and derivatives have been disclosed to promote wound healing in soft tissues such as skin and mucosa (WO 99/43344). The use of enamel matrix proteins or enamel matrix derivatives for dentin regeneration has not, to the inventors' knowledge, been reported previously.

Exposure of vital dental pulp is a common complication, either accidentally or by design, during normal dental restorative and prosthodontic procedures and also following crown fractures, trauma, and caries. Traditionally minimal exposures of dental pulp are treated either by applying a $Ca(OH)_2$ paste or a filling material (e.g. composites) directly onto the pulp to induce superficial necrosis sometimes followed by sclerotisation and reactive dentin formation deeper down in the pulp tissue. This strategy, however, seldom restores a mineralised barrier between the pulp and the restorative material(s) and in frequent cases the dental pulp becomes inflamed and has to be treated by pulpectomy (removal of the entire pulp) or pulpotomy (removal of a large portion of the pulp) and subsequent endodontic filling with synthetic materials (e.g. guttapercha). In the cases where a larger part of the pulp is exposed treatment always includes a pulpectomy and endodontic filling.

Endodontically treated teeth lose their blood supply, innervation and ability to produce reactive hard tissue (secondary dentin). As a result, these teeth become brittle and are also more prone to masticatory induced trauma and grave caries (due to lack of sensibility patients do not feel pain). This makes the lifespan of endodontically treated teeth much

shorter than that of vital teeth and thus less useful as pillars for prosthodontic tooth replacements.

If a way to induce new dentin formation that efficiently can cover and seal off exposed dental pulp could be found, it would constitute a major breakthrough in conservative dentistry.

SUMMARY OF THE INVENTION

The present invention is based on the surprising finding that enamel matrix, enamel matrix derivatives or enamel matrix proteins (collectively termed "active enamel substance" in the following) are able to induce dentin formation in dental pulp cells.

Accordingly, the invention relates to the use of a preparation of an active enamel substance for the preparation of a pharmaceutical composition for the formation or regeneration of dentin following dental procedures involving exposure of vital dental pulp tissue.

In another aspect, the invention relates to a method of promoting the formation or regeneration of dentin following dental procedures involving exposure of vital dental pulp tissue, the method comprising applying an effective amount of an active enamel substance on exposed vital dental pulp tissue after dental procedures.

The observation that enamel matrix proteins are capable of inducing dentin formation in vital pulp tissue was first made in experiments with developing teeth where pulp cells were accidentally exposed to enamel matrix during a surgical procedure to remove the enamel organ from developing teeth in rats. The findings have later been confirmed by repeated experiments both in rats and pigs. Furthermore, the inventors have developed a tooth trauma model in adult pigs which confirms that the use of an enamel matrix protein composition is highly efficient in inducing hard tissue formation in the exposed dental pulp. That enamel proteins promotes dentin formation in developed pulp tissue is surprising since these proteins are only naturally present in developing teeth and never present in other tissues or in mature dental tissues (i.e. in adults or children of more than about 12 years of age).

Without wishing to be limited to any particular theory, it is assumed that because the action of these molecules during tooth formation is associated with the onset of mineralisation of both dentin and enamel, these proteins are likely to be part of a mechanism for dental hard tissue formation. Based on the present findings, it would appear that even though enamel matrix proteins are completely removed from dental tissue upon tooth development, cells in the adult dental pulp retain their ability to respond to these proteins by inducing dormant developmental processes for dentin formation.

It would appear that the mechanism by which enamel matrix proteins induce dentin formation differs significantly from the mechanism inducing cementum formation reported by L. Hammarström, *J. Clin. Periodontol. 24*, 1997, pp. 658-668, where application of enamel matrix proteins to experimental cavities drilled in exposed root surfaces resulted in the formation of cementum when the experimental teeth were replanted in contact with periodontal ligament. In the case reported by Hammarström, formation of cementum occurred directly on the surface of already formed hard tissue. According to the present invention, on the other hand, ectopic dentin is formed, i.e. enamel matrix proteins are able to stimulate formation of dentin at sites where no pre-existing hard tissues are found.

DETAILED DESCRIPTION OF THE INVENTION

Teeth are composed of three hard (mineralized) tissues, namely enamel, dentin and cementum, and centrally located soft tissue, called dental pulp. The dental pulp is mainly composed of fibroblasts and contains blood vessels and nerves. The dental pulp of a fully formed tooth (mature tooth) is enclosed by dentin on all sides except for a small foramen at the apical end of the root. Dentin is the major dental tissue and is formed by specific cells termed odontoblasts. Different types of dentin have been distinguished. Most of the dentin is formed during tooth development and is termed primary dentin. After the period of tooth development, some dentin formation continues but at a markedly slower rate. The dentin formed after tooth development is termed secondary dentin. Certain events such as occurrence of dental caries, cavity preparation and trauma may induce an accelerated form of dentin termed reactive, reparative or tertiary dentin.

Morphologically, primary and secondary dentin is a non-cellular dental, mineralized tissue containing narrow canals called dentinal tubules extending from the pulp towards the periphery. Next to the pulp, there is a thin non-mineralized layer termed predentin.

Reparative dentin may occasionally have a more irregular morphology with more irregular distribution of dentinal tubules. Occasionally, dentin may contain enclosed cells in which case it is termed osteodentin. For a further discussion of dental morphology, reference is made to A.R. Ten Cate, *Oral Histology. Development, Structure and Function*, 5th Ed., Mosby 1998, pp. 150-196).

As indicated above, it has surprisingly been found that the active enamel substance is capable of promoting the formation of reactive dentin in dental pulp tissue even in mature teeth (i.e. teeth of adults or children of more than about 12 years of age) which are not naturally exposed to enamel matrix or proteins present therein. In a particular embodiment, the invention therefore relates to the use of the active enamel substance for the regeneration of secondary dentin in vital dental pulp tissue. The regeneration of secondary dentin is particularly advantageous as secondary dentin has a structure closely resembling that of primary dentin, and the application of active enamel substance therefore participates in the restoration of dental pulp with a structural organisation giving it similar properties to the dental pulp originally formed in the tooth.

Alternatively, however, the active enamel substance is also useful for the formation of reparative dentin or osteodentin in vital dental pulp tissue, thereby ensuring a quick reparative process in the dental pulp although this results in the formation of dentin of a somewhat poorer quality than primary or secondary dentin as the composition and structure does not resemble that of primary dentin. The reparative process mainly occurs upon major dental trauma involving exposure of dental pulp, or upon major dental procedures such as pulpotomy or pulpectomy.

As indicated above, the use of the active enamel substance is particularly useful for promoting dentin formation or regeneration in vital dental pulp tissue in erupted teeth in which enamel matrix proteins are not naturally present in sufficient amounts to provide a healing effect following dental procedures. This is the case both for adults and children whose permanent teeth have developed.

The ability of the active enamel substance to promote or induce formation for dentin may also be exploited in association with cavity preparation, especially deep cavities where it is desirable to increase the thickness of the dentinal wall to avoid or reduce the risk of pulpal exposure, to avoid or reduce the exposure of the pulp to toxic or irritating sub-

stances, or to reduce or eliminate the hypersensitivity that often appears after dental treatment.

According to the present use, the preparation of active enamel substance is preferably applied onto dental pulp before application of a filling material following dental procedures involving exposure of vital dental pulp tissue. Pharmaceutical compositions suitable for such application are discussed below in further detail.

Enamel matrix, enamel matrix derivatives and enamel matrix proteins

Enamel matrix is a precursor to enamel and may be obtained from any relevant natural source, i.e. a mammal in which teeth are under development. A suitable source is developing teeth from slaughtered animals such as, e.g., calves, pigs or lambs. Another source is for example fish skin.

Enamel matrix can be prepared from developing teeth as described previously (EP-B-0 337 967 and EP-B-0 263 086). The enamel matrix is scraped off and enamel matrix derivatives are prepared, e.g. by extraction with aqueous solution such as a buffer, a dilute acid or base or a water/solvent mixture, followed by size exclusion, desalting or other purification steps, optionally followed by freeze-drying. Enzymes may be deactivated by treatment with heat or solvents, in which case the derivatives may be stored in liquid form without freeze-drying.

In the present context, enamel matrix derivatives are derivatives of enamel matrix which include one or several enamel matrix proteins or parts of such proteins, produced naturally by alternate splicing or processing, or by either enzymatic or chemical cleavage of a natural length protein, or by synthesis of polypeptides in vitro or in vivo (recombinant DNA methods or cultivation of diploid cells). Enamel matrix protein derivatives also include enamel matrix related polypeptides or proteins. The polypeptides or proteins may be bound to a suitable biodegradable carrier molecule, such as polyamino acids or polysaccharides, or combinations thereof. Furthermore, the term enamel matrix derivatives also encompasses synthetic analogous substances.

Proteins are biological macromolecules constituted by amino acid residues linked together by peptide bonds. Proteins, as linear polymers of amino acids, are also called polypep-

tides. Typically, proteins have 20-800 amino acid residues and hence have molecular weights in the range of from about 6,000 to about several hundred thousand Daltons or more. Small proteins (of less than about 20 amino acids) are usually called peptides or oligopeptides.

Enamel matrix proteins are proteins which normally are present in enamel matrix, i.e. the precursor for enamel (Ten Cate: Oral Histology, 1994; Robinson: Eur. J. Oral Science, Jan. 1998, 106 Suppl. 1:282-91), or proteins which can be obtained by cleavage of such proteins. In general such proteins have a molecular weight below 120,000 daltons and include amelogenins, non-amelogenins, proline-rich non-amelogenins, amelins (ameloblastin, sheathlin), tuftelins, dentinsialoprotein (DSP) or dentinsialophosphoprotein (DSPP).

Examples of proteins for use according to the invention are amelogenins, proline-rich non-amelogenins, tuftelin, tuft proteins, serum proteins, salivary proteins, amelin, ameloblastin, sheathlin, and derivatives thereof, and mixtures thereof. A preparation containing an active enamel substance for use according to the invention may also contain at least two of the aforementioned proteinaceous substances. A commercial product mainly comprising amelogenins is marketed as EMDOGAIN® (Biora AB).

In general, the major proteins of an enamel matrix are known as amelogenins. They constitute about 90% w/w of the matrix proteins. The remaining 10% w/w includes proline-rich non-amelogenins, tuftelin, tuft proteins, serum proteins and at least one salivary protein; however, other proteins may also be present such as, e.g., amelin (ameloblastin, sheathlin) which have been identified in association with enamel matrix. Furthermore, the various proteins may be synthesised and/or processed in several different sizes (i.e. different molecular weights). Thus, the dominating proteins in enamel matrix, amelogenins, have been found to exist in several different sizes which together form supramolecular aggregates. They are markedly hydrophobic substances which under physiologically conditions form aggregates. They may carry or be carriers for other proteins or peptides.

Other protein substances are also contemplated to be suitable for use according to the present invention. Examples include proteins such as proline-rich proteins and polyproline. Other examples of substances which are contemplated to be suitable for use

according to the present invention are aggregates of such proteins, of enamel matrix derivatives and/or of enamel matrix proteins as well as metabolites of enamel matrix, enamel matrix derivatives and enamel matrix proteins. The metabolites may be of any size ranging from the size of proteins to that of short peptides.

As mentioned above, the proteins, polypeptides or peptides for use according to the invention typically have a molecular weight of at the most about 120 kDa such as, e.g., at the most 100 kDa, 90 kDa, 80 kDa, 70 kDa or 60 kDa as determined by SDS Page electrophoresis.

The proteins for use according to the invention are normally presented in the form of a preparation, wherein the protein content of the active enamel substance in the preparation is in a range of from about 0.05% w/w to 100% w/w such as, e.g., about 5-99% w/w, about 10-95% w/w, about 15-90% w/w, about 20-90% w/w, about 30-90% w/w, about 40-85% w/w, about 50-80% w/w, about 60-70% w/w, about 70-90% w/w, or about 80-90% w/w.

A preparation of an active enamel substance for use according to the invention may also contain a mixture of active enamel substances with different molecular weights.

The proteins of an enamel matrix can be divided into a high molecular weight part and a low molecular weight part, and it has been found that a well-defined fraction of enamel matrix proteins possesses valuable properties with respect to treatment of periodontal defects (i.e. periodontal wounds). This fraction contains acetic acid extractable proteins generally referred to as amelogenins and constitutes the low molecular weight part of an enamel matrix (cf. EP-B-0 337 967 and EP-B-0 263 086).

As discussed above the low molecular weight part of an enamel matrix has a suitable activity for inducing binding between hard tissues in periodontal defects. In the present context, however, the active proteins are not restricted to the low molecular weight part of an enamel matrix. At present, preferred proteins include enamel matrix proteins such as amelogenin, amelin, tuftelin, DSP, etc. with molecular weights (as measured in vitro with SDS-PAGE) below about 60,000 daltons.

Accordingly, it is contemplated that the active enamel substance for use according to the invention has a molecular weight of up to about 40,000 such as, e.g. a molecular weight of between about 5,000 and about 25,000.

Within the scope of the present invention are also peptides as described in WO 97/02730, i.e. peptides which comprise at least one sequence element selected from the group consisting of the tetrapeptides DGEA (Asp-Gly-Glu-Ala), VTKG (Val-Thr-Lys-Gly), EKGE (Glu-Lys-Gly-Glu) and DKGE (Asp-Lys-Gly-Glu) and which further comprise an amino acid sequence from which a consecutive string of 20 amino acids is identical to a degree of at least 80% with a string of amino acids having the same length selected from the group consisting of the amino acid sequence shown in SEQ ID NO:1 and a sequence consisting of amino acids 1 to 103 of SEQ ID NO:1 and amino acids 6 to 324 of SEQ ID NO:2.

By the term "sequence identity" is meant the identity in sequence of amino acids in the match with respect to identity and position of the amino acids of the peptides. A gap is counted as non-identity for one or more amino acids as appropriate.

Such peptides may comprise from 6 to 300 amino acids, e.g. at least 20 amino acids, at least 30 amino acids, such as at least 60 amino acids, at least 90 amino acids, at least 120 amino acids, at least 150 amino acids or at least 200 amino acids.

A method for the isolation of enamel matrix proteins involves extraction of the proteins and removal of calcium and phosphate ions from solubilised hydroxyapatite by a suitable method, e.g. gel filtration, dialysis or ultrafiltration (see e.g. Janson, J-C & Rydén, L. (Eds.), Protein purification, VCH Publishers 1989 and Harris, ELV & Angal, S., Protein purification methods - A practical approach, IRL Press, Oxford 1990).

A typical lyophilised protein preparation may mainly or exclusively up to 70-90% contain amelogenins with a molecular weight (MW) between 40,000 and 5,000 daltons, the 10-30% being made up of smaller peptides, salts and residual water. The main protein bands are at 20 kDa, 12-14 kDa and around 5 kDa as determined by SDS-PAGE.

By separating the proteins, e.g. by precipitation, ion-exchange chromatography, preparative electrophoresis, gel permeation chromatography, reversed phase chromatography or affinity chromatography, the different molecular weight amelogenins can be purified.

The combination of molecular weight amelogenins may be varied, from a dominating 20 kDa compound to an aggregate of amelogenins with many different molecular weights between 40 and 5 kDa, and to a dominating 5 kDa compound. Other enamel matrix proteins such as amelin, tuftelin or proteolytic enzymes normally found in enamel matrix, can be added and carried by the amelogenin aggregate.

As an alternative source of the enamel matrix derivatives or proteins one may also use generally applicable synthetic routes well-known for a person skilled in the art or use cultivated cells or bacteria modified by recombinant DNA-techniques (see, e.g., Sambrook, J. et al.: Molecular Cloning, Cold Spring Harbor Laboratory Press, 1989).

Physico-chemical properties of enamel matrix, enamel matrix derivatives and enamel matrix proteins

In general the enamel matrix, enamel matrix derivatives and enamel matrix proteins are hydrophobic substances, i.e. less soluble in water especially at increased temperatures. In general, these proteins are soluble at non-physiological pH values and at a low temperature such as about 4-20°C, while they will aggregate and precipitate at body temperature (35-37°C) and neutral pH.

The enamel matrix, enamel matrix derivatives and/or enamel matrix proteins for use according to the invention also include an active enamel substance, wherein at least a part of the active enamel substance is in the form of aggregates or after application in vivo is capable of forming aggregates. The particle size of the aggregates is in a range of from about 20 nm to about 1 μm.

The enamel matrix, enamel matrix derivatives and enamel matrix proteins have also been observed (by the present inventors) to posses bioadhesive properties, i.e. they have an ability to adhere firmly to tissue surfaces. These properties are most valuable in connection with endodontic treatment not least because they ensure a fast and intimate contact

between the enamel matrix proteins and the dentin-producing odontoblasts so as to facilitate the process of dental root regeneration.

Theories with respect to mechanism of action

Enamel matrix is an example of an extracellular protein matrix which adheres to mineral surfaces as well as to proteinaceous surfaces. At physiological pH and temperature the amelogenins present in enamel matrix form an insoluble supra-molecular aggregate (Fincham et al. in J. Struct. Biol. 1994 March-April; 112(2):103-9 and in J. Struct. Biol. 1995 July-August; 115(1):50-9), which is gradually degraded by proteolytic enzymes (occurs both in vivo and in vitro provided that the proteases have not been subjected to inactivation).

The recent observation that enamel matrix is formed and temporarily present during root and root cementum formation can explain how application of enamel matrix, enamel matrix derivatives and/or enamel matrix proteins promotes the regeneration of periodontal tissue. However, the observation underlying the present invention that enamel matrix, enamel matrix derivatives and/or enamel matrix proteins also exert a positive effect on formation or regeneration of endodontic tissue, i.e. dental pulp tissue, is very surprising.

A preparation of the active enamel substance is normally formulated as a pharmaceutical composition. Such a composition may of course consist of the proteinaceous preparation or it may further comprise a pharmaceutically acceptable diluent or excipient. The diluent may typically be water in which the active enamel substance is dissolved before application to endodontic tissue.

Pharmaceutical compositions

In the following examples of suitable compositions containing the active enamel substance are given.

For the administration to an individual (an animal or a human) the enamel matrix, enamel matrix derivatives and/or enamel matrix proteins (in the following also denoted "active enamel substance") and/or a preparation thereof are preferably formulated into a pharma-

ceutical composition containing the active enamel substance and, optionally, one or more pharmaceutically acceptable excipients.

The compositions may be in form of, e.g., solid, semi-solid or liquid compositions such as, e.g.,

powders, granules, granulates, capsules, agarose or chitosan beads, tablets, pellets, microcapsules, microspheres, nanoparticles, or freeze-dried powders, granules, granulates or pellets,

gels, hydrogels, pastes,

solutions, dispersions, suspensions, emulsions, mixtures,

kits containing e.g. two separate containers, wherein the first one of the containers contains the active enamel substance optionally admixed with other active drug substance(s) and/or pharmaceutically acceptable excipients and the second container containing a suitable medium intended to be added to the first container before use in order to obtain a ready-to-use composition.

The compositions may be formulated according to conventional pharmaceutical practice, see, e.g., "Remington's Pharmaceutical Sciences" and "Encyclopedia of Pharmaceutical Technology", edited by Swarbrick, J. & J. C. Boylan, Marcel Dekker, Inc., New York, 1988.

Apart from the active enamel substance, a pharmaceutical composition for use according to the invention may comprise pharmaceutically acceptable excipients.

A pharmaceutically acceptable excipient is a substance which is substantially harmless to the individual to which the composition is to be administered. Such an excipient normally fulfils the requirements given by the national health authorities. Official pharmacopoeias such as e.g. the British Pharmacopoeia, the United States of America Pharmacopoeia and The European Pharmacopoeia set standards for pharmaceutically acceptable excipients.

The choice of pharmaceutically acceptable excipient(s) in a composition for use according to the invention and the optimum concentration thereof cannot generally be predicted and must be determined on the basis of an experimental evaluation of the final composition. However, a person skilled in the art of pharmaceutical formulation can find guidance in e.g., "Remington's Pharmaceutical Sciences", 18th Edition, Mack Publishing Company, Easton, 1990.

The pharmaceutically acceptable excipients may include solvents, buffering agents, preservatives, chelating agents, antioxidants, stabilisers, suspending agents and gel-forming agents.

Examples of solvents are e.g. water, alcohols, or other hydrophilic or etheric solvents such as weak acids with a pH of about 5.5-6.0 facilitating the subsequent application of filling materials in the tooth.

Examples of buffering agents are e.g. citric acid, acetic acid, tartaric acid, lactic acid, hydrogenphosphoric acid, diethylamine etc.

Suitable examples of preservatives for use in compositions are parabens, such as methyl, ethyl, propyl p-hydroxybenzoate, butylparaben, isobutylparaben, isopropylparaben, potassium sorbate, sorbic acid, benzoic acid, methyl benzoate, phenoxyethanol, bronopol, bronidox, MDM hydantoin, iodopropynyl butylcarbamate, benzalconium chloride, and benzylalcohol, or mixtures of preservatives.

Examples of antioxidants are butylated hydroxy anisole (BHA), ascorbic acid and derivatives thereof, tocopherol and derivatives thereof, cysteine, and mixtures thereof.

Examples of suspending agents are e.g. celluloses and cellulose derivatives such as, e.g., carboxymethyl cellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, microcrystalline cellulose (e.g. Avicel® RC 591), carragheenan, acacia gum, arabic gum, tragacanth, and mixtures thereof.

Examples of gel bases or viscosity-increasing agents are liquid paraffin, polyethylene, fatty oils, colloidal silica or aluminium, zinc soaps, glycerol, propylene glycol, tragacanth, carboxyvinyl polymers, magnesium-aluminium silicates, Carbopol®, hydrophilic polymers

such as, e.g. starch or cellulose derivatives such as, e.g., carboxymethylcellulose, hydroxyethylcellulose and other cellulose derivatives, water-swellable hydrocolloids, carragenans, hyaluronates, and alginates including propylene glycol alginate.

Examples of powder components are: alginate, collagen or lactose. Normally, powders intended for application on dental pulps must be sterile and the particles present must be micronized.

Examples of other excipients are polymers such as carmelose, sodium carmelose, hydroxypropylmethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, pectin, xanthan gum, locust bean gum, acacia gum, gelatin, carbomer, emulsifiers like vitamin E, glyceryl stearates, cetanyl glucoside, collagen, carrageenan, hyaluronates and alginates and chitosans.

Suitable compositions for use according to the invention may also be presented in the form of suspensions, emulsions or dispersions. Such compositions contains the active enamel substance in admixture with a dispersing or wetting agent, suspending agent, and/or one or more preservatives and other pharmaceutically acceptable excipients. Suitable dispersing or wetting agents are, for example, naturally occurring phosphatides, e.g., lecithin, or soybean lecithin; condensation products of ethylene oxide with e.g. a fatty acid, a long chain aliphatic alcohol, or a partial ester derived from fatty acids and a hexitol or a hexitol anhydride, for example polyoxyethylene stearate, polyoxyethylene sorbitol monooleate, polyoxyethylene sorbitan monooleate, etc.

Dosages of enamel matrix, enamel matrix derivatives and enamel matrix proteins

In a pharmaceutical composition for use according to the invention, an active enamel substance is generally present in a concentration ranging from about 0.01% to about 99.9% w/w. The amount of composition applied will normally result in an amount of total protein per cm^2 area of dental pulp corresponding to from about 0.005 mg/mm^2 to about 5 mg/mm^2 such as from about 0.01 mg/mm^2 to about 3 mg/mm^2.

In those cases where the active enamel substance is administered in the form of a liquid composition, the concentration of the active enamel substance in the composition is in a range corresponding to from about 0.01 to about 50 mg/ml, e.g. from about 0.1 to about

30 mg/ml. Higher concentrations are in some cases desirable and can also be obtained such as a concentration of at least about 100 mg/ml. Defect areas in dental pulp in humans typically have a size of about 5-10 x 2-4 x 5-10 mm corresponding to about 200 µl and normally at the most about 0.5-1 ml such as about 0.2-0.3 ml per tooth is applied of a composition having a concentration of about 1-40 mg total protein/ml such as, e.g., 5-30 mg/ml is applied. 0.2-0.3 mg/ml corresponds to about 6 mg protein per 25-100 mm^2 or about 0.1 mg/mm^2 if calculated only on root surface. Normally an excessive volume is applied to cover the affected surfaces adequately. Even a multilayer would only require a small fraction of the above-mentioned amounts.

The present invention is further described in the following examples which are not in any way intended to limit the scope of the invention as claimed.

EXPERIMENTAL SECTION

Example 1

Six adult (18 months of age or older) Göttingen minipigs (available from Møllegård, Denmark) were anesthesized with Dormicum (a general anesthetic available from Roche, Switzerland) and also locally anesthesized by injection of Xylocain and adrenalin. The pulps of permanent maxillary premolars and molars (a total of 36 teeth) were exposed in buccal class V cavities using a sterilised round steel burr (No. 12) with saline spray. The most coronal part of the pulp was then removed to make a pulp wound of with an area of more than 2 square millimetres. The vitality of the pulp was demonstrated by abundant bleeding that was brought under control using sterile cotton pellets. After bleeding had stopped, an enamel matrix derivative (EMD, Emdogain®, available from Biora AB) or Ca(OH)2 paste (Dycal Dentine, available from Dentsply DeTrey, Switzerland) as control, were applied directly onto the exposed pulp. The cavities were then sealed with a commercially available glass ionomer filling (GC Fuji II, Fuji Co., Japan) in a procedure mimicking ordinary clinical situations.

After two or four weeks, the animals were sacrificed and the experimental teeth were extracted and embedded in paraffin, and histological sections were stained with hematoxylin and eosin. Microscopy of the histological sections revealed a thick dentin-like closure of the pulp chamber adjacent to the filling material after four weeks in the location where

EMD had been applied (Figs. 1-3). In controls without EMD no or only rudimentary dentin formation was observed and none of the control teeth exhibited complete closures of the pulp chamber (Figs. 4-6).

CLAIMS

1. Use of a preparation of an active enamel substance for the preparation of a pharmaceutical composition for the formation or regeneration of dentin following dental procedures involving exposure of vital dental pulp tissue.

2. Use according to claim 1 for the regeneration of secondary dentin in vital dental pulp tissue.

3. Use according to claim 1 for the formation of reparative dentin or osteodentin in vital dental pulp tissue.

4. Use according to claim 1 for promoting dentin formation in vital dental pulp tissue in erupted teeth.

5. Use according to claim 1, wherein the preparation of active enamel substance is applied onto dental pulp before application of a filling material following dental procedures involving exposure of vital dental pulp tissue.

6. Use according to any of claims 1-5, wherein the active enamel substance is enamel matrix, enamel matrix derivatives and/or enamel matrix proteins.

7. Use according to claim 6, wherein the active enamel substance is selected from the group consisting of enamelins, amelogenins, non-amelogenins, proline-rich non-amelogenins, amelins (ameloblastin, sheathlin), tuftelins, DSP, DSPP, and derivatives thereof and mixtures thereof.

8. Use according to claim 6, wherein the active enamel substance has a molecular weight of at the most about 120 kDa such as, e.g, at the most 100 kDa, 90 kDa, 80 kDa, 70 kDa or 60 kDa as determined by SDS-PAGE electrophoresis.

9. Use according to any of claims 6-8, wherein the preparation of an active enamel substance contains a mixture of active enamel substances with different molecular weights.

10. Use according to claim 9, wherein the preparation of an active enamel substance comprises at least two substances selected from the group consisting of amelogenins, proline-rich non-amelogenins, enamelins, tuftelin, tuft proteins, serum proteins, salivary proteins, amelin, ameloblastin, sheathlin, DSP, DSPP, and derivatives thereof.

11. Use according to any of claims 6-10, wherein the active enamel substance has a molecular weight of up to about 40,000.

12. Use according to claim 11, wherein the active enamel substance has a molecular weight of between about 5,000 and about 25,000.

13. Use according to claim 12, wherein the major part of the active enamel substance has a molecular weight of about 20 kDa.

14. Use according to any of claims 6-13, wherein at least a part of the active enamel substance is in the form of aggregates or after application in vivo is capable of forming aggregates.

15. Use according to claim 14, wherein the aggregates have a particle size of from about 20 nm to about 1 μm.

16. Use according to any of claims 6-15, wherein the protein content of the active enamel substance in the preparation is in a range of from about 0.05% w/w to 100% w/w such as, e.g., about 5-99% w/w, about 10-95% w/w, about 15-90% w/w, about 20-90% w/w, about 30-90% w/w, about 40-85% w/w, about 50-80% w/w, about 60-70% w/w, about 70-90% w/w, or about 80-90% w/w.

17. Use according to any of claims 1-16, wherein the preparation of the active enamel substance is in freeze-dried form.

18. Use according to any of claims 1-16, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

19. Use according to claim 18, wherein the pharmaceutically acceptable excipient is propylene glycol alginate.

20. Use according to claim 18, wherein the pharmaceutically acceptable excipient is hyaluronic acid or salts or derivatives thereof.

21. Use according to any of claims 1-20 of EMDOGAIN® or any proteins or peptides contained therein for the formation or regeneration of dentin following dental procedures involving exposure of vital dental pulp tissue.

22. A method of promoting the formation or regeneration of dentin following dental procedures involving exposure of vital dental pulp tissue, the method comprising applying an effective amount of an active enamel substance on exposed vital dental pulp tissue after dental procedures.

23. The method of claim 22, wherein the application of the active enamel substance is followed by application of a filling material.

24. The method of claim 22, which is for the regeneration of secondary dentin in vital dental pulp tissue.

25. The method of claim 22, which is for the formation of reparative dentin or osteodentin in vital dental pulp tissue.

26. The method of claim 22, which is for promoting dentin formation in vital dental pulp tissue in erupted teeth.

27. The method of any of claims 22-26, wherein the active enamel substance is applied in an amount of total protein per cm^2 area of affected dental pulp tissue, corresponding to from about 0.005 mg/cm^2 to about 5 mg/cm^2, such as from about 0.01 mg/cm^2 to about 3 mg/cm^2.

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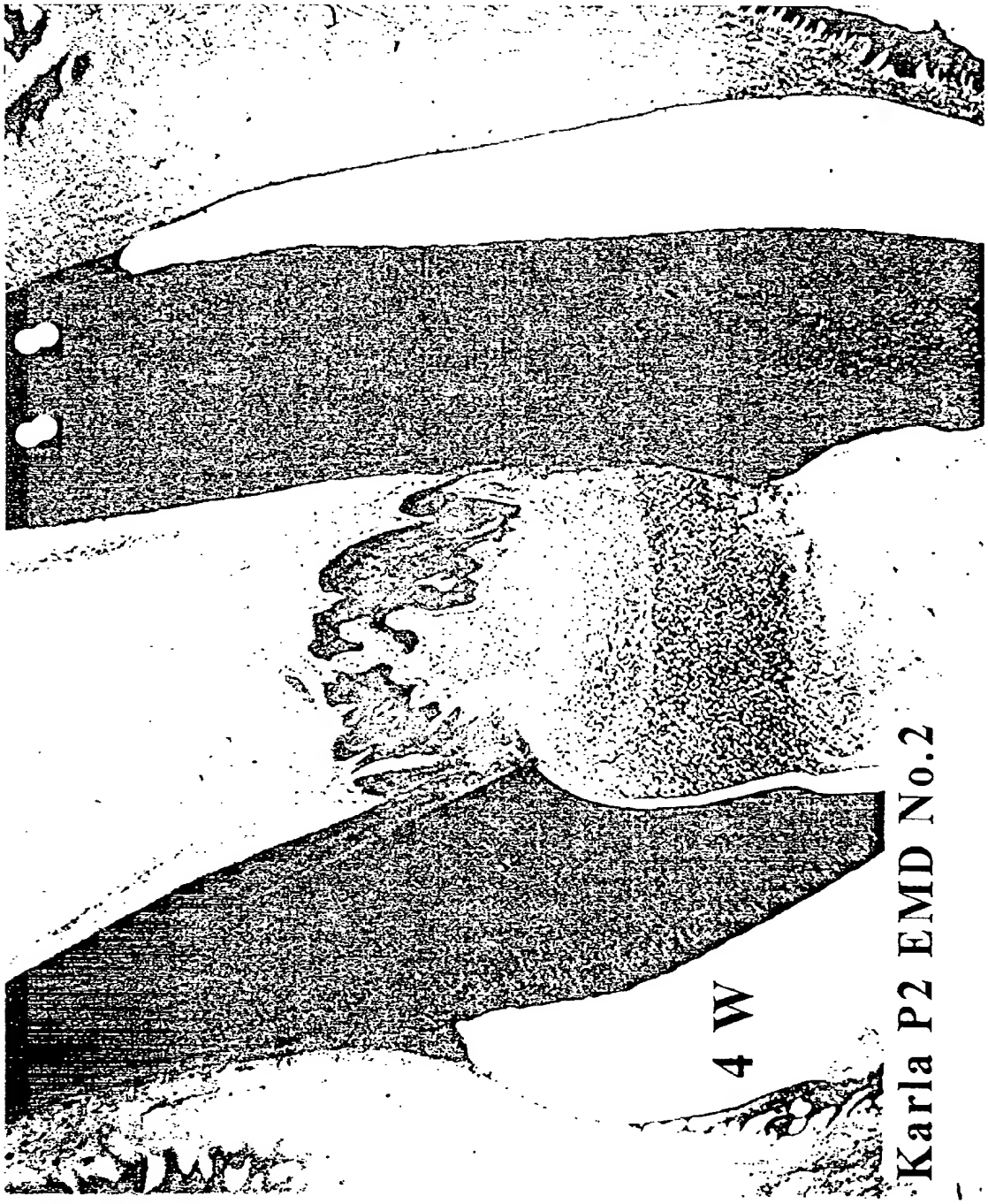

Fig. 1

Modtaget
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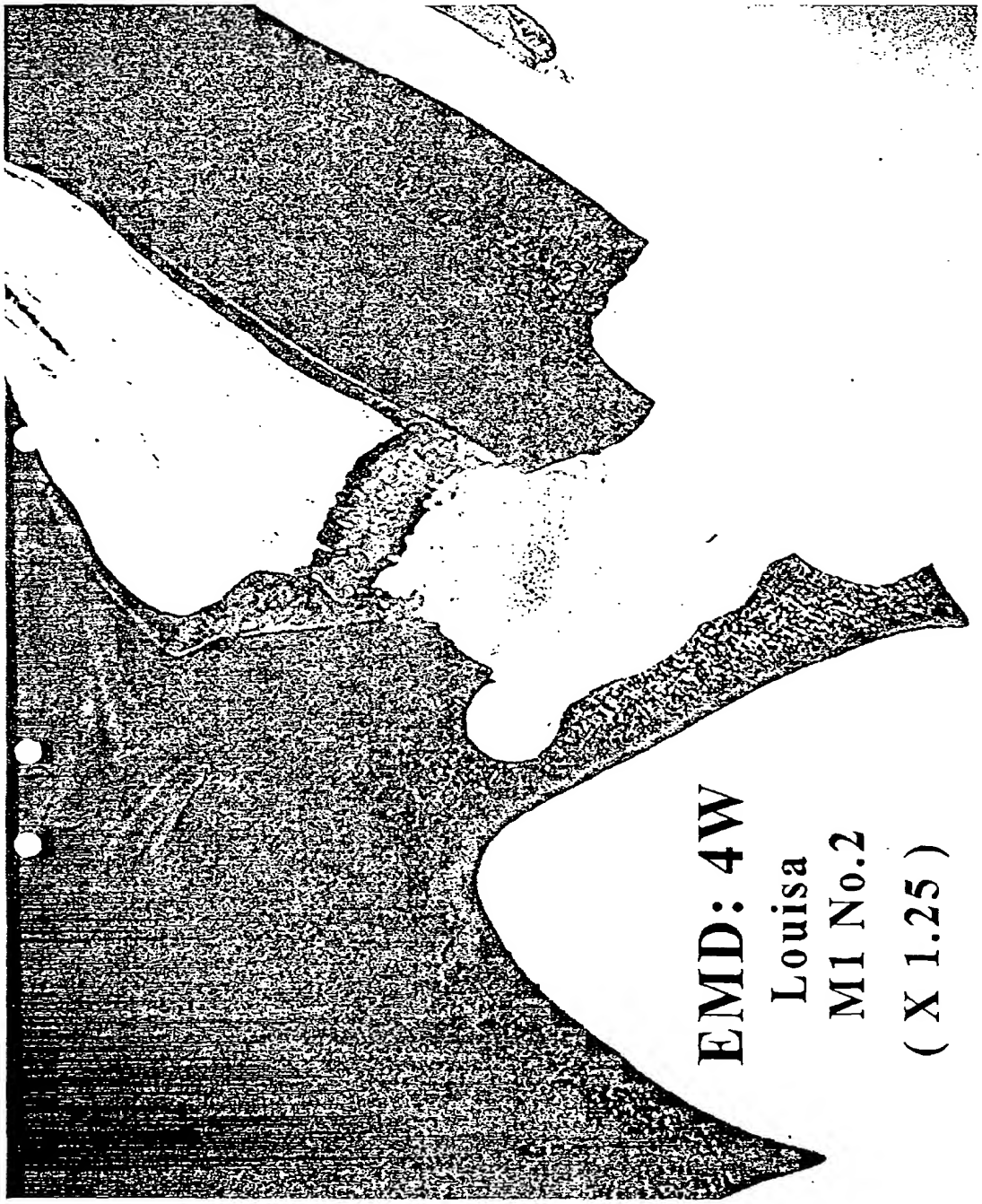

Fig. 2

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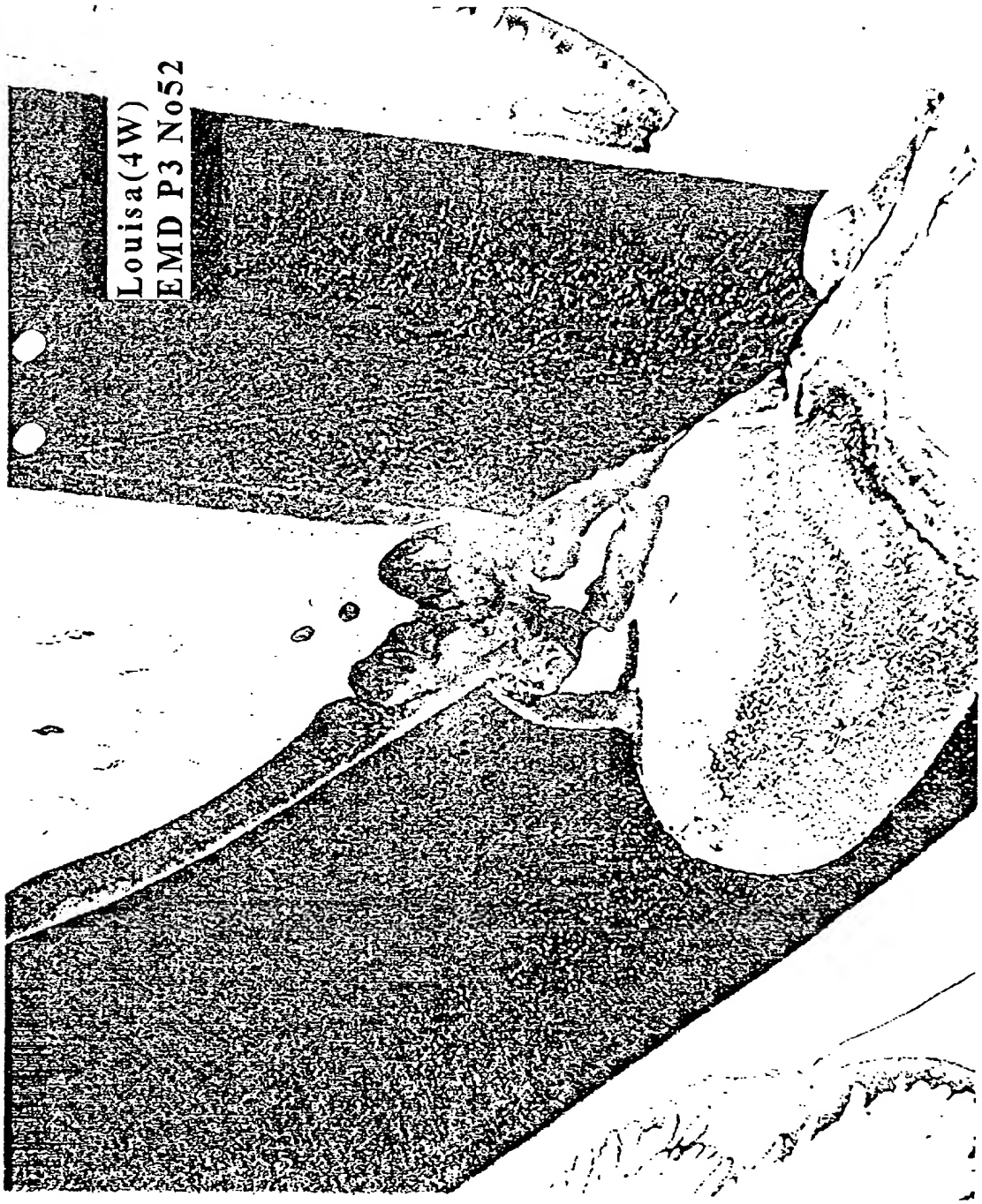

Fig. 3

Fig. 3

Modtaget
20 JUNI 2000
PVS

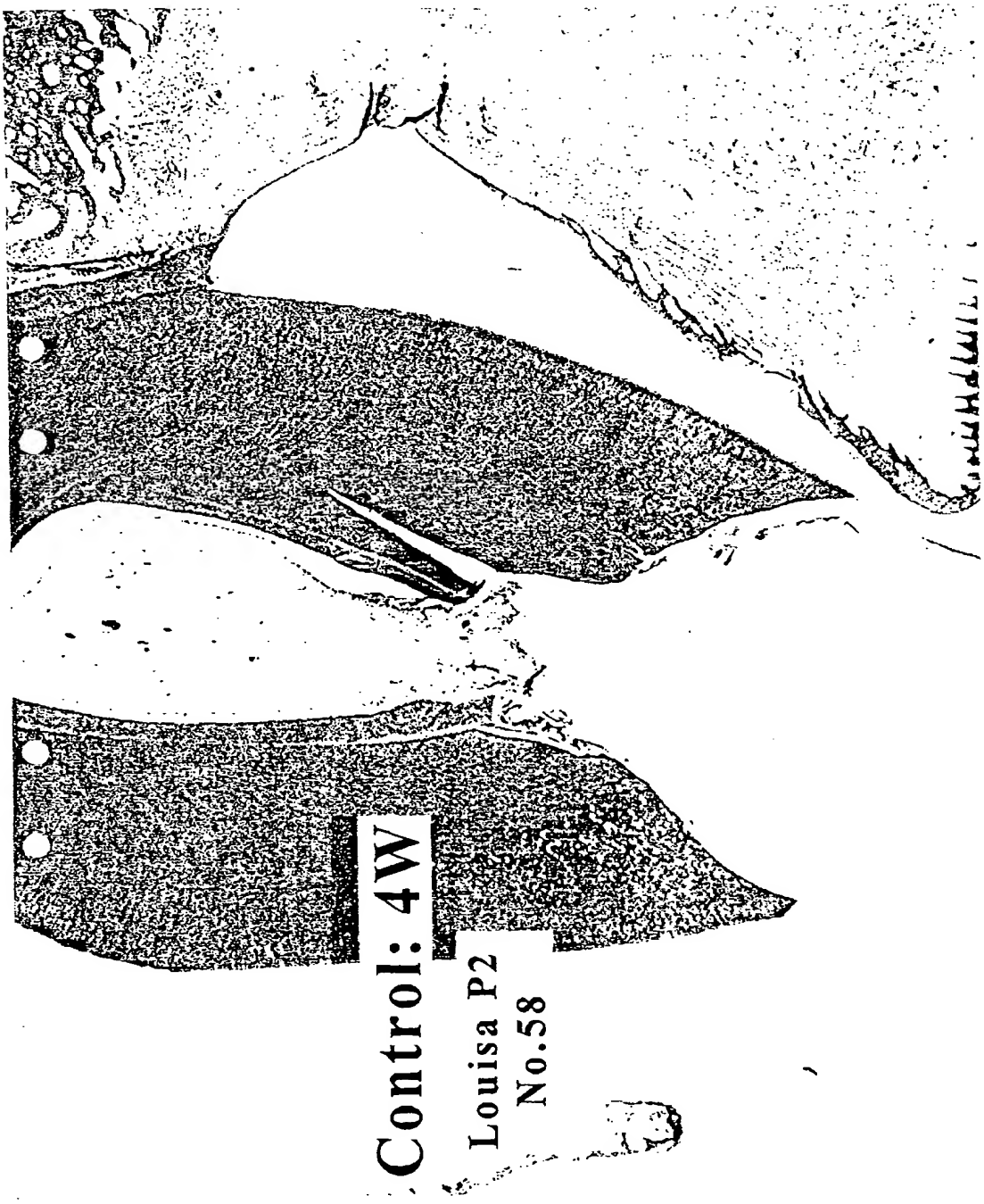

Fig. 4

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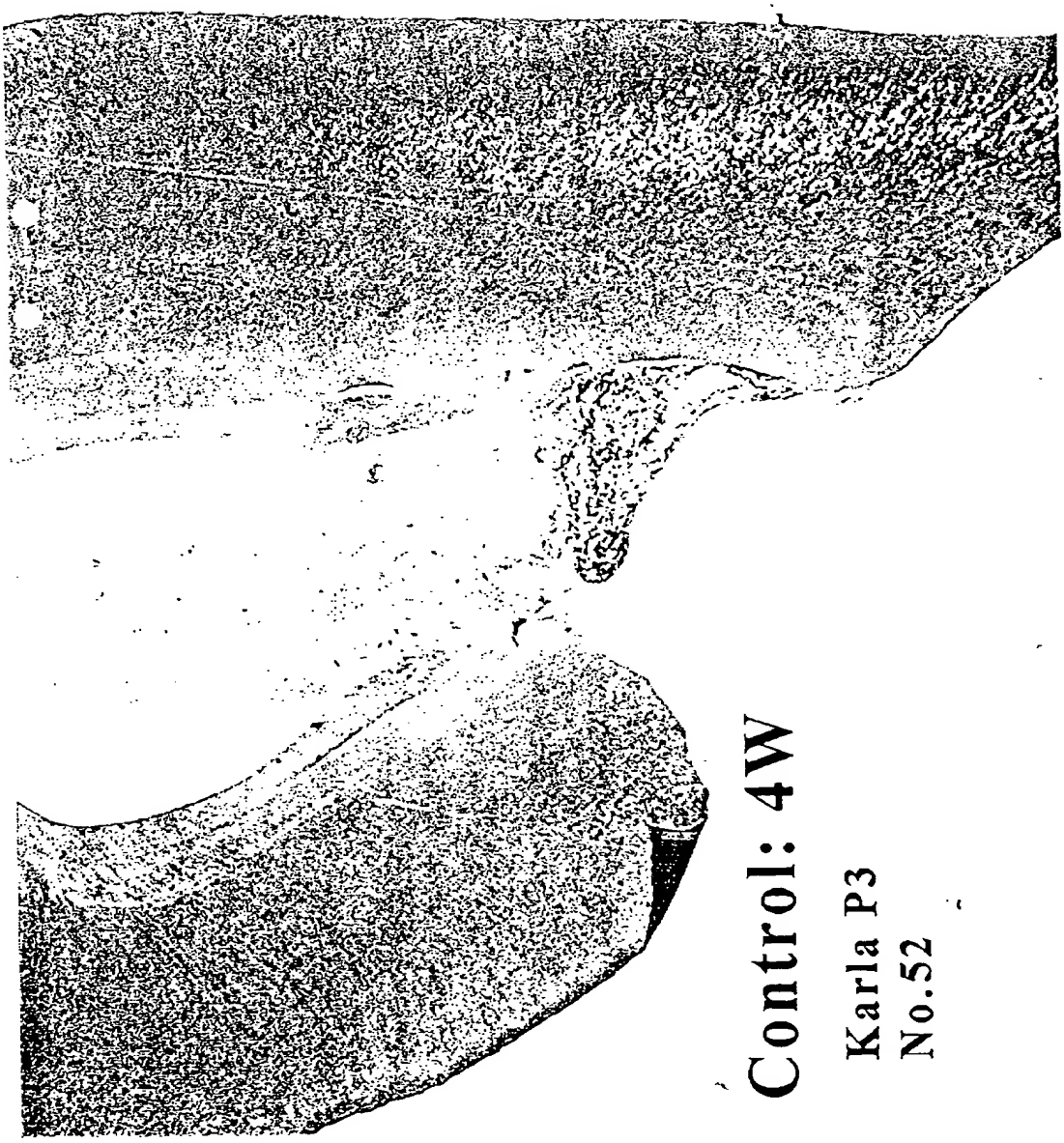

Fig. 5

Modtaget
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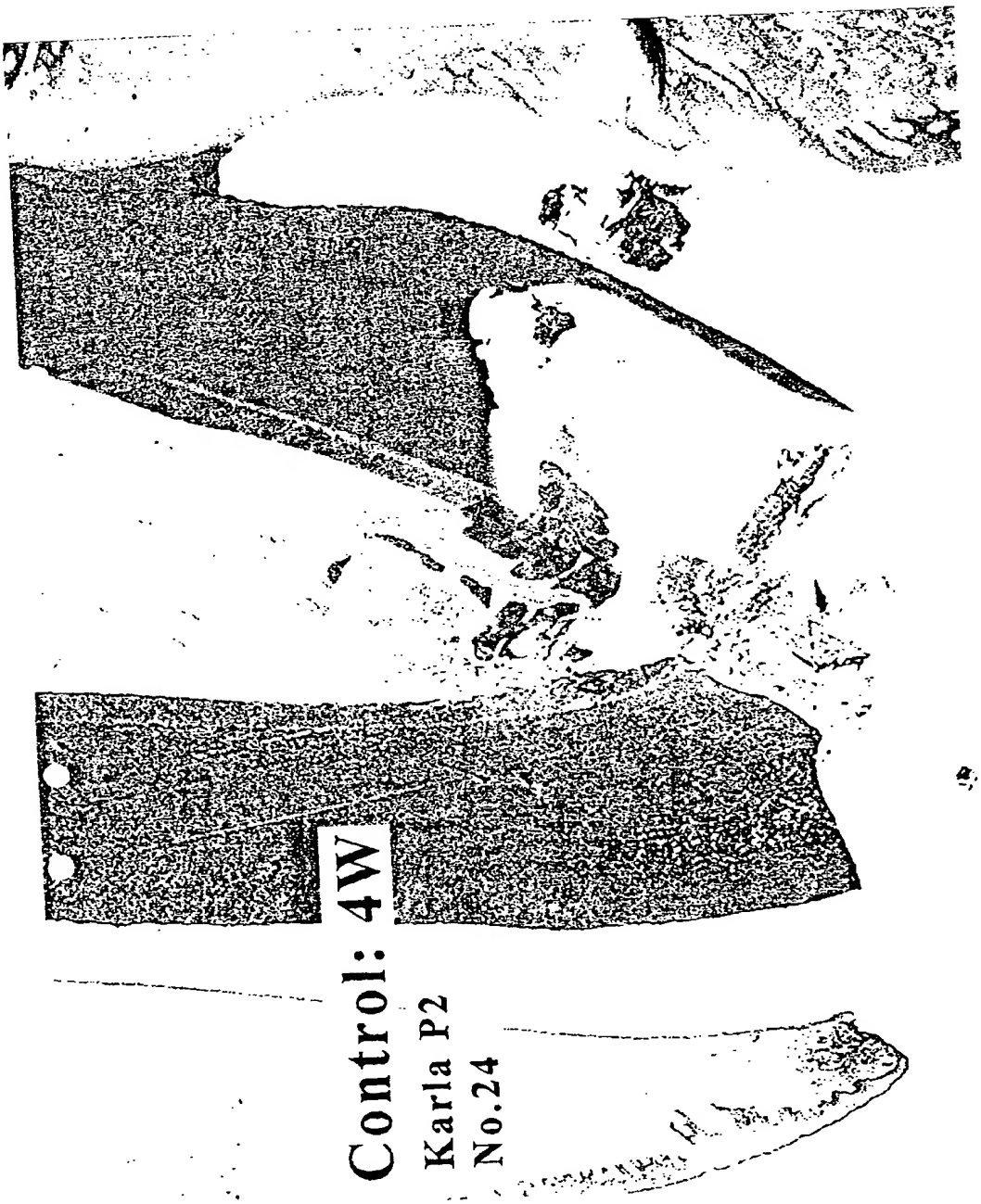

Fig. 6